A Proposal to Estimate the Relative Reproductive Success of Hatchery and Naturally Produced Steelhead in the Wenatchee River Basin

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Introduction and Background

Hatcheries are one of the main tools that have been used to mitigate for salmon losses caused by the construction and operation of the Columbia River hydropower system. Historically, the goal of most hatcheries was to provide fish for harvest and mitigate for hydro related impacts and habitat loss. Over the last ~100 years, biologists, fisheries managers, fisherman, and the public have been frequently concerned about the status of the Pacific Northwest's salmon populations (Lichatowich 1999). This concern culminated in the 1990's, when the National Marine Fisheries Service listed 26 (now 27) Evolutionary Significant Units (ESUs) of west coast Pacific salmon as threatened or endangered under the Endangered Species Act. As concerns for the conservation of wild salmon grew in the late 20th Century, the intent of many hatchery programs changed from providing fish for harvest and mitigation to conserving and rebuilding natural populations. As a result, hatcheries now are a large component in most conservation or recovery programs, particularly for ESUs in the Interior Columbia River Basin. The use of hatcheries to conserve wild salmon is controversial, however, due in part to concerns about the genetic impacts of even well intentioned hatchery supplementation on wild populations (Waples and Drake 2004).

Genetic risks associated with hatchery supplementation include the potential for increased inbreeding (Ryman and Laikre 1991; Ryman et al. 1995; Wang and Ryman 2001), outbreeding depression (e.g., Gharrett and Smoker 1991) and domestication selection (Ford 2002). The potential seriousness of these phenomena is reinforced by a long history of studies showing that hatchery fish often reproduce poorly in the wild when compared to natural origin fish (reviewed by Berejikian and Ford 2004, Araki et al. 2008). Hatchery steelhead, in particular, have been found to have very low relative reproductive success in a several studies (Chilcote et al. 1986; Leider et al. 1990; Chilcote 2003; Kostow et al. 2003; McLean et al. 2003; Araki et al. 2007a; Araki et al. 2007b).

Evaluating the relative reproductive success of Wenatchee River steelhead is particularly important for several reasons because supplementation is being used as a significant component of the recovery strategy for this population and might increase in the future. Indeed, the Rock Island Habitat Conservation Plans (HCP) requires Chelan PUD to "investigate the natural spawning success of hatchery reared steelhead relative to wild steelhead" (section 8.5.3 of CCPUD 2002). In addition, Upper Columbia steelhead have a very large 'gap' between current productivity and productivity needed to meet viability goals (ICTRT 2007). This large gap may be due, at least in part, to the large proportion of hatchery fish in this population. Understanding the relative reproductive success of hatchery steelhead in this population is therefore particularly important for evaluating the recovery potential for this population.

It is important to note that the study we are proposing is only part of what is required to evaluate the effects of supplementation on wild steelhead in the Upper Columbia. In particular, estimating the relative fitness of hatchery fish is important, but it is not sufficient for evaluating the effectiveness of supplementation. Long-term studies comparing the demographic performance of supplemented and unsupplemented populations are also important, and may provide a more complete understanding of the overall impacts of supplementation. For example, several studies have found widespread negative correlations between natural population productivity and intensity of hatchery production (Chilcote 2003; Nickelson 2003; Hoekstra et al. 2007). The results of these studies, which primarily focus on segregated and often non-local hatchery populations, are consistent with the finding that such hatchery populations often have very low relative fitness. Similar analyses of the long-term demographic effects of supplementation should also be conducted for Upper Columbia steelhead to complement shorter-term studies of relative fitness (Murdoch and Peven 2005).

Starting in 2007, NOAA Fisheries has funded a project to collect of DNA samples from steelhead migrating upstream of Tumwater Dam and their juvenile progeny in order to start a full pedigree-based relative reproductive success project for this population. This proposal is a continuation of that project that (1) directly measure the relative reproductive success of hatchery and natural-origin steelhead in the natural environment using a DNA pedigree approach, and (2) determine the degree to which any differences in reproductive success between hatchery and natural steelhead can be explained by measurable biological characteristics such as run timing, morphology, spawn timing, or spawning location.

Life History of Steelhead in the Wenatchee Basin.

Upstream Migration

Run timing of steelhead has been monitored using video recording equipment at Tumwater Dam since 1998 (Figure 1). Of those fish migrating above Tumwater Dam, almost 80% migrate upstream between June 15 and December 15 (Figure 2). A smaller upstream migration (~20%) occurs in February through May immediately prior to spawning. Run timing comparison between hatchery and wild steelhead has not been conducted because the external mark on hatchery-origin fish was a visual implant elastomer (VIE) tag that is not visible using the videotaping technique rather than an adipose fin clip, which would be recognizable videotapes. Although, portions of the current releases of hatchery steelhead (~50%) are adipose fin clipped, the level is still inadequate to make comparisons.

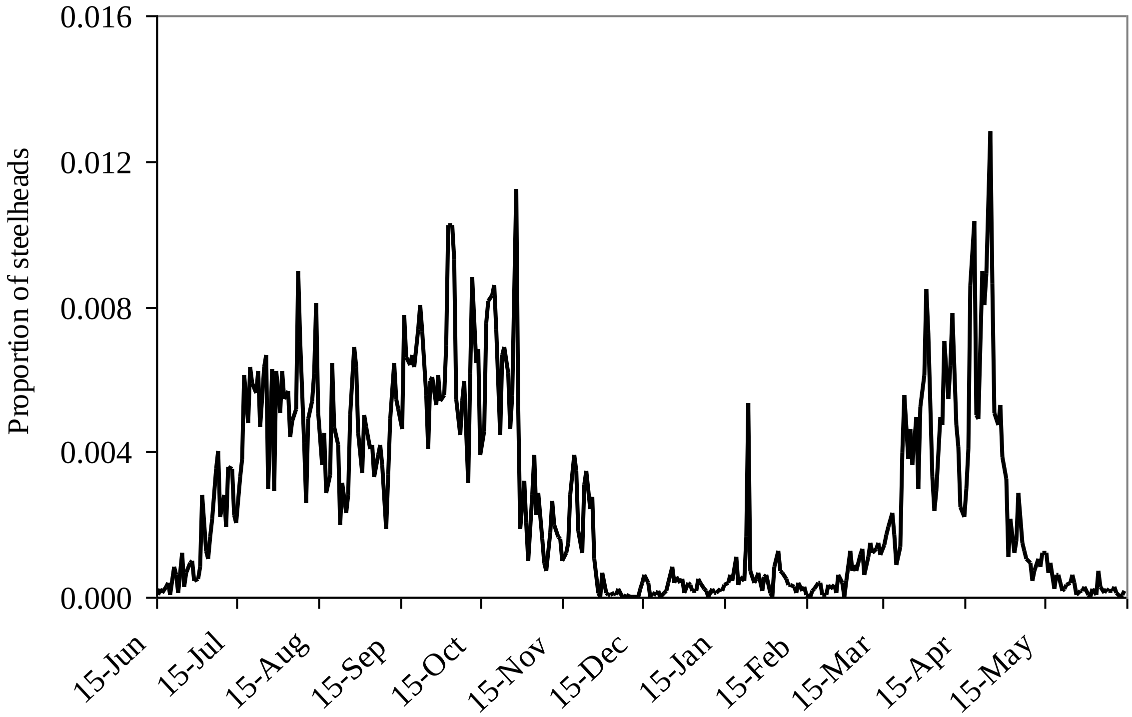

Figure 1 - Mean daily proportion of Wenatchee steelhead migrating upstream of Tumwater Dam, 1998-2006.

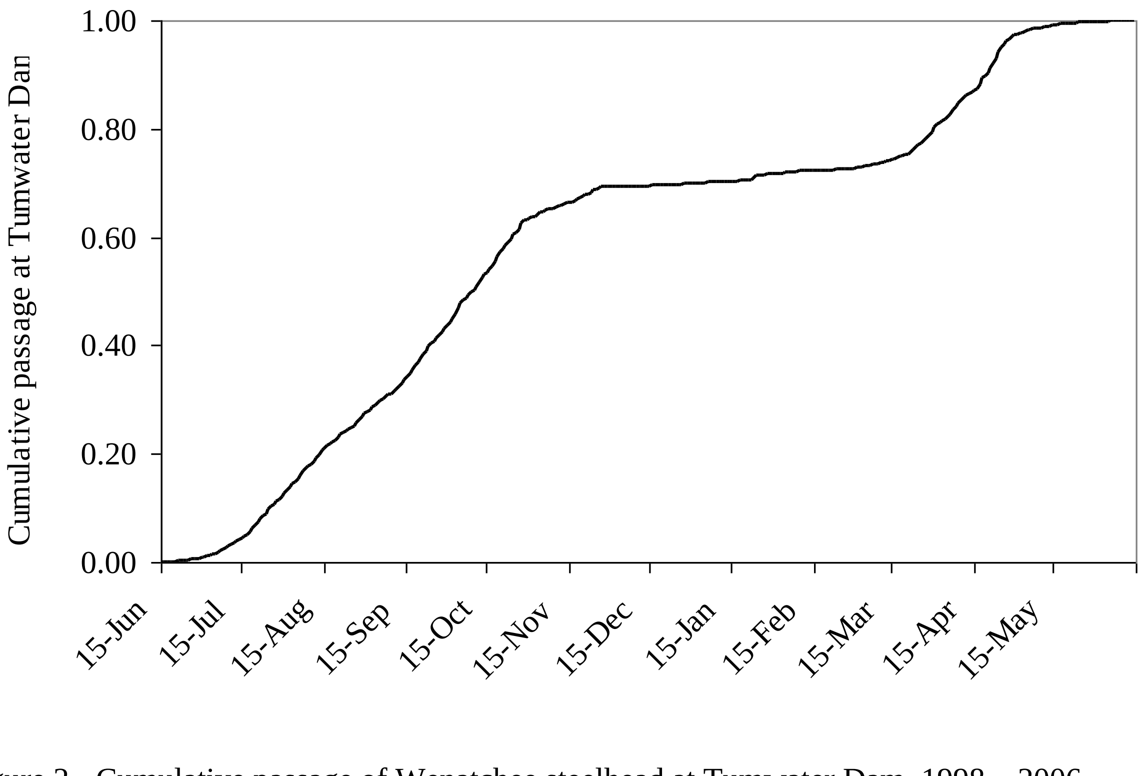

Figure 2 - Cumulative passage of Wenatchee steelhead at Tumwater Dam, 1998 – 2006.

Escapement Estimates

Run escapement upstream of Tumwater Dam has been estimated since 1998 (i.e., 1999 spawners). Based on 10 years of data, 15 June has been identified as the start of the next brood year. For reporting purposes, any steelhead passing upstream of Tumwater Dam on or after 15 June will be counted in the next brood year (e.g., 15 June 2007 - 14 June 2008 = 2008 brood). Estimated steelhead escapement abundance and proportion of the total run escapement upstream of Tumwater Dam has increased since 1999, likely as a result of a change in the release location of juvenile hatchery fish (i.e., shifted upstream of Tumwater Dam) and a combination of improved mainstem passage and ocean survival (Table 1). As previously discussed, the proportion of hatchery and naturally produced fish upstream of Tumwater Dam is unknown because most hatchery fish during this time period were not adipose fin clipped. Gender was determined by secondary sexual characteristics (i.e., kype, coloration, body shape), but will be determined using a portable ultrasound beginning with the 2009 brood.

Table 1 - Steelhead escapement and estimated sex composition monitored and estimated at Tumwater Dam and the proportion of the total run escapement upstream of Tumwater Dam, 1999-2008). Fish collected for hatchery broodstock are included.

Brood Year	Tumwater Dam [2]				Estimated total run escapement into Wenatchee Basin [1]
	Female	Male	Total	Proportion of total run	
1999	--	--	179	0.34	524
2000	--	--	541	0.39	1,371
2001	--	--	858	0.43	2,016
2002	644	1,081	1,725	0.29	5,851
2003	1,151	686	1,837	0.60	3,042
2004	875	1,058	1,933	0.57	3,406
2005	1,651	998	2,649	0.63	4,216
2006	550	584	1,134	0.43	2,628
2007	356	334	690	0.59	1,171
2008	510	888	1,398	0.52	2,709
2009	919	766	1,685	0.73	2,322

1 A. Murdoch, WDFW, unpublished data. Wenatchee run escapement estimates are based on the difference between Rock Island and Rocky Reach Dam counts adjusted using radio telemetry information, 1 June – 31 May (English et al. 2001 and 2003).

2 Run escapement to Tumwater Dam based on the number fish at Tumwater Dam, 15 June – 14 June.

Spawning

Historical run escapement estimates derived from Columbia River dam counts (i.e., Rock Island minus Rocky Reach) may not provide an accurate estimate of the Wenatchee spawning population because steelhead, between Rock Island and Rocky Reach Dams, may be harvested, suffer prespawn mortality, or spawn in locations other than the

Wenatchee Basin. Radio telemetry data suggest that 85% of the steelhead that were detected in the area between Rock Island and Rocky Reach dams during the time of spawning, entered the Wenatchee River (English et al. 2003). In 2001, steelhead spawning ground surveys were initiated in the Wenatchee Basin to collect data on the spawning population (e.g., distribution, abundance, and spawn timing). Spawning generally occurs between the end of March and early June. Lack of marks recognizable at a distance on most hatchery-origin steelhead, variable water clarity during surveys, and low recovery rates of carcasses, have prevented any direct comparisons of life history characteristics between hatchery and naturally produced steelhead other than those that can be performed on hatchery program broodstock.

Smolt Emigration

Potential size overlap between age classes requires an estimate of age for each individual juvenile fish in order to assign them to the correct brood year. Age estimates based on scales suggest naturally produced steelhead emigrate from the Wenatchee River (i.e., March through June) between age-1 and age-4 (Figure 3), and the most common smolt age class is age-2 (Figure 4;WDFW, unpublished data). Scale samples from steelhead smolts collected in the Upper Columbia River have been reported to underestimate age when compared to ages estimated from otoliths (Peven et al. 1994). Because we are not proposing to collect otoliths to compare age estimates, brood year assignment may be problematic.

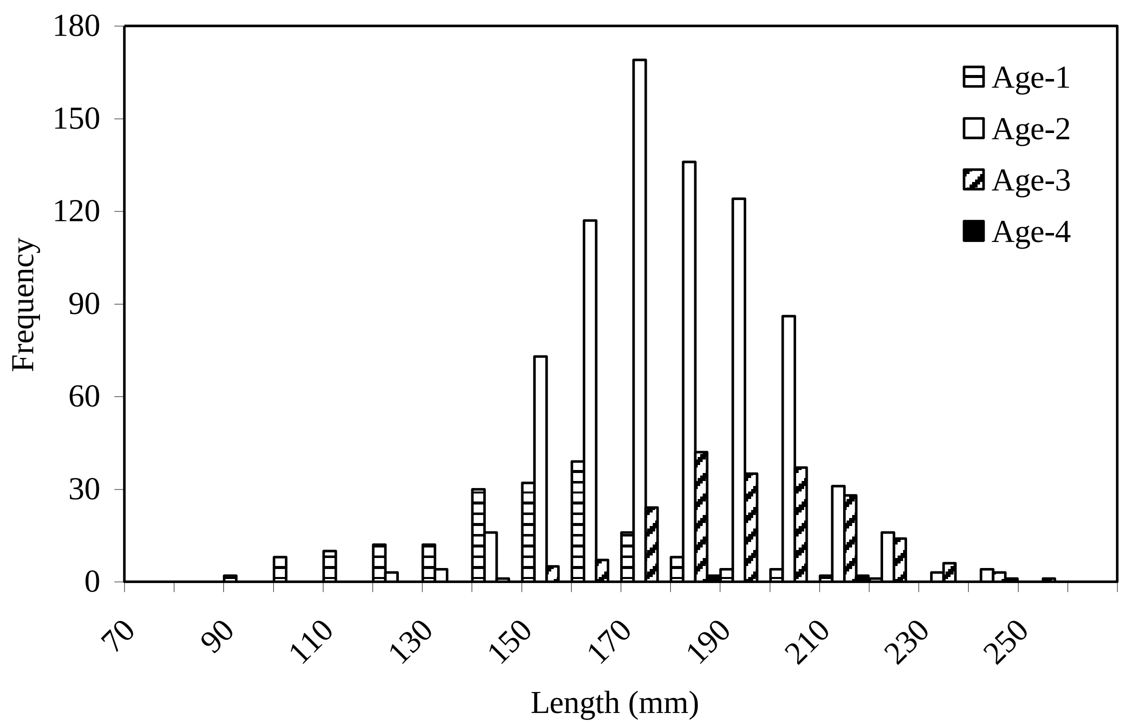

Figure 3 - Length frequency of juvenile steelhead captured at the lower Wenatchee River (rkm 9.6) smolt trap, 2001 – 2006.

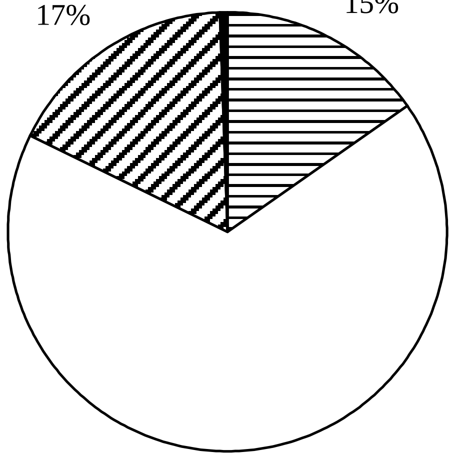

Figure 4 - Age composition of naturally produced steelhead smolts captured at the Lower Wenatchee smolt trap, 2001 – 2006.

Information on the life histories of steelhead in the upper Wenatchee Basin is available from video recordings at Tumwater Dam, mainstem Columbia River dam counts, and redd counts. Each of these data sources have limited ability to distinguish natural and hatchery-origin steelhead, determine age-at-return, or other characteristics that could be important to reproductive success and long-term persistence of Wenatchee Basin steelhead. Data collected from hatchery program broodstock may not be representative of the population at large and does not provide any information regarding the reproductive success of fish spawning in the natural environment. The study proposal below would greatly improve our understanding of the life history characteristics of natural and hatchery-origin steelhead and the reproductive success of both components of the population.

Methods

The methods described below outline the proposed methodology for estimate the relative reproductive success of hatchery and naturally produced steelhead. During the course of the study, we will also complete the necessary steps to fulfill Objective 2 and 3 of the Hatchery Monitoring and Evaluation Plan. Details regarding these aspects of the proposal can be found in Attachment A (Objective 2), Attachment B (Objective 3) and Murdoch and Peven (2005).

Task 1 – Collect DNA tissues from all or an adequate sample of adults

In a manner consistent with an ongoing study of Wenatchee spring Chinook relative reproductive success (Murdoch et al. 2007), we will sample steelhead as they return to locations upstream of Tumwater Dam to spawn. We intend to sample four complete brood years of spawners (2008 – 2011). Our experimental design has its greatest statistical power when a large fraction of all potential spawners are sampled. Adults will be collected in a trap at Tumwater Dam beginning in June and ending the following May. The trap will be operated 24 hours a day, 7 days a week in coordination with the ongoing reproductive success study for spring Chinook salmon.

Tumwater trap operation

During periods when low numbers of fish are expected to migrate upstream of Tumwater Dam (< 20 fish/day) the trap will be operated passively (e.g., November – March). When the trap is operated in the passive configuration, fish will be collected in a large holding chamber with continuous supply of water. Because most fish movement occurs during the day, the trap will be checked a minimum every evening. Nontarget species will be released immediately upstream of the dam. When large numbers of fish (i.e., sockeye and summer Chinook salmon) are migrating through Tumwater Dam the trap will be operated in the active configuration during the day (16 – 18 h) and passive configuration only at night after migration rates have decreased to a nominal level. Fish will be held in a live box for a maximum of 4 h before they are processed and released. While the trap is in the active configuration all fish other than spring Chinook and steelhead will be released upstream, unless collected by WDFW or Yakama Nation for other ongoing hatchery programs.

Biological sampling

Every fish captured would be handled in a manner consistent with standard fish handling protocols and conditions for handling ESA listed steelhead prescribed in ESA permit 1395. Each steelhead trapped will be measured (fork and post-orbital to hypural plate length) to the nearest cm, weighed to the nearest gram, scales collected, determine gender based on secondary sexual characteristics or a portable ultrasound device, and will have a small piece (~0.5 cm^2) of caudal fin removed for genetic analysis. Each fish will also be scanned for internal tags and classified as either hatchery produced or naturally produced, based on the presence or absence of a hatchery mark (adipose fin clip, visible elastomer tag, or eroded fins). However, some unmarked hatchery fish will also be captured (i.e., lost tag). In which case, scales collected from each fish will be used for both aging and for confirming either hatchery or natural origin. Depending on run sizes, we will sample between 500-2,000 adults per year.

All steelhead will also be implanted with a passive integrated transponder (PIT) tag (females in body cavity; males in pelvic girdle). The exceptions are those fish already PIT tagged from various other studies. Both hatchery and naturally produced summer steelhead may exhibit extensive prespawning migratory patterns. Recaptures of PIT tagged adults at PIT tag interrogation sites downstream of Tumwater Dam can be removed from the pool of potential parents and increase the accuracy of estimates of reproductive success. In-stream PIT tag antenna arrays are deployed in most major spawning areas in the Wenatchee Basin and depending on detection rates may also allow assessments of reproductive success at the tributary level (e.g., Chiwawa River and Nason Creek).

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Task 2 – Collect DNA tissues from a representative sample of naturally produced juveniles

A systematic sample of up to 1,500 parr and 1,500 smolts will be collected from each brood year, consistent with sample size goals of ongoing studies. Juvenile sampling will occur in conjunction with existing activities already ongoing in the Wenatchee Basin. No additional juvenile fish will be collected specifically for this study. Naturally produced fish will be distinguished from hatchery produced fish by the absence of a mark or tag, eroded fins or scale pattern. Fish will be weighed to the nearest 0.1 g, measured to the nearest mm (fork length), scale sampled, and a small portion of the distal portion of the dorsal lobe of the caudal fin (~0.1 cm^2) will be clipped for DNA analysis. A representative sample of both parr (*N* = 750) and smolts (*N* = 750) will be analyzed.

Juvenile DNA Collection – Age-1 Parr

The majority of juvenile steelhead emigrate from the Wenatchee Basin as age-2 or older smolts (85%). We propose collecting DNA samples from age-1 steelhead parr collected as part of the Integrated Status and Effectiveness Monitoring Program (ISEMP) funded by BPA (Project # 2003-017-00) and Chelan County PUD. As part of ongoing activities, WDFW personnel will capture up to 500 steelhead parr in each of the three major spawning areas (*N* = 1,500) upstream of Tumwater Dam (i.e., Nason Creek, Chiwawa River and Wenatchee River). Sampling would be conducted between July and October using a combination of angling and snorkel/seining (i.e., snerding) capture techniques (Figure 5, Table 3). All fish captured will be PIT tagged by Chelan County PUD personnel to include fork length and weight.

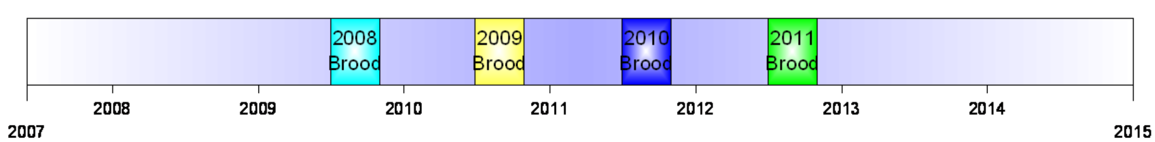

Figure 5. Sampling timeline for age-1 steelhead parr in the Wenatchee Basin.

After each steelhead has been tagged, WDFW or Chelan PUD personnel will scale sample and collect a tissue sample from each steelhead. In order to estimate age and brood year, each juvenile steelhead will be scale sampled, which will be read by WDFW Scale Lab in Olympia. Collecting scale samples from juvenile salmonids is a routine activity conducted whenever juvenile fish are sampled (i.e., smolt traps). Potential impacts from scale sampling (e.g., increased disease susceptibility) to individual fish would likely be small, but may be reduced if early in the study we determine that at the time of collection sufficient separation in length for each age class exists. Furthermore, PIT tagging each juvenile should eliminate the probability of double sampling the same individual. Additionally, residual hatchery steelhead captured during these activities will be PIT tagged, DNA sampled, and included in the data set of potential parents (i.e., Tumwater Dam data). A smolt trap is also operated on the White River and may be used to collect samples from parr (N < 100) that otherwise may not be sampled under existing activities.

Table 2 - DNA sampling schedule and approximate number of samples to be collected and analyzed.

Life stage	Location	N per year	Sample Period (year)					
Parr	Nason	500	2009	2010	2011	2012		
	Chiwawa	500	2009	2010	2011	2012		
	Wenatchee	500	2009	2010	2011	2012		
Smolt	Nason	500	2009	2010	2011	2012	2013	2014
	Chiwawa	500	2009	2010	2011	2012	2013	2014
	Wenatchee	500	2009	2010	2011	2012	2013	2014

Juvenile DNA Collection – Smolts

Tissue samples of steelhead smolts will be collected from existing rotary smolts traps operated in the Chiwawa River, Nason Creek, and lower Wenatchee River. As part of these ongoing activities, steelhead smolts will be weighed to the nearest 0.1 g, measured (fork length) to the nearest mm, scale sampled, and PIT tagged. At the beginning of the study only steelhead smolts from brood years in which adults were sampled (determined from scale samples) will be included in the analysis. In subsequent years, depending on the duration of the project all steelhead smolts captured will be included in the analysis. Currently under the ISEMP project, Chelan County PUD personnel PIT tag fish at the Chiwawa and lower Wenatchee smolt traps and Yakama Nation personnel PIT tag fish at the Nason Creek smolt trap (Figure 6, Table 2). WDFW personnel operate both the Chiwawa and lower Wenatchee smolt traps and will provide additional staff required to collect scale and DNA samples if required. WDFW will also provide staff, upon request to assist the Yakama Nation personnel operating and PIT tagging steelhead smolts at the Nason Creek smolt trap.

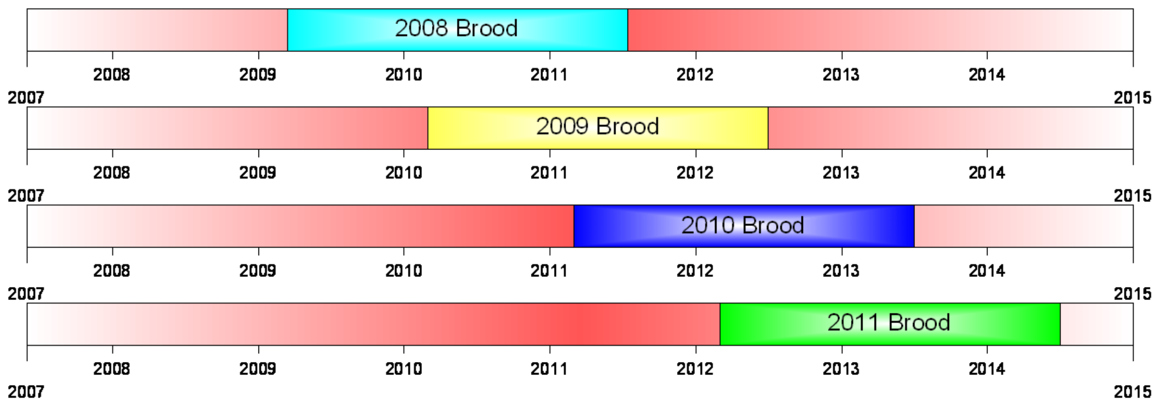

Figure 6. Sampling timeline for steelhead smolts in the Wenatchee Basin.

Age Estimation of Juvenile Steelhead

Incorrectly assigning age or brood year to juvenile steelhead would decrease the parental assignment rate and subsequently reduce the ability to make inferences regarding reproductive success. Validation of scale pattern analysis will be required in order to correctly assign a juvenile fish to its parent brood year. This source of error has not been adequately addressed in previous studies. The freshwater ages of naturally produced adult Wenatchee steelhead broodstock, estimated from otoliths, may be used to assess the potential bias in age estimates based on scales. Although the specific procedures to accomplish scale pattern validation without using otoliths (e.g., lethal sampling) have not been developed, the use of scale patterns for juvenile fish (progeny) correctly assigned to adults (parents) may be suitable.

Task 3 – Estimate fitness, relative reproductive success, and selection gradients

We will use genetic parentage analysis to estimate the number of sampled progeny (juvenile and adult) produced by each potential spawner sampled at Tumwater Dam. This approach has been successfully applied to several salmonid species, including steelhead (McLean et al. 2003; Seamons 2004; Seamons et al. 2004; Araki et al. 2007a; Araki et al. 2007b), coho (Ford et al. 2006), Chinook (Murdoch et al. 2007), pink (Dickerson et al. 2005), and Atlantic salmon (McGinnity 1997).

Microsatellite genotyping - Genomic DNA will be extracted from fin clips using a QIAgen DNA tissue extraction kit, eluted into a 96-well sample plate, and quantified using a FLX 800 Microplate Fluorescence reader (Bio-Tek Instruments, Winooski, Vermont). All original DNA extractions as well as the working stocks of DNA will be stored at -20^{o}C until needed. Unused portions of fin-clips will appropriately cataloged and stored. Individuals will be genotyped at 16 previously developed di- and tetranucleotide repeat microsatellite loci (Table 3). PCR products and in-lane size standards (GeneScan 500) will be resolved using an ABI3100 capillary electrophoresis system (Applied Biosystems, Inc., Foster City, California). Individual genotypes will be scored using Genotyper software (Applied Biosystems, Inc., Foster City, CA).

Genotyping error rate per locus will be estimated by re-amplifying and re-scoring microsatellite loci for a random subset of individuals, and calculating the number of alleles mis-scored over the total number of alleles observed at each locus.

Parentage assignment – Parentage assignments will be made using the likelihood methods of Meagher and Thompson (1986) and Gerber et al. (2000) as implemented in the program FAMOZ (Gerber et al. 2003). Each individual in a sample of progeny will be tested against all potential pairs of parents and a log of odds (LOD) score will be calculated for each potential parent pair/offspring triplet as the log of the ratio of the probability of a parent pair/offspring relationship compared to the probability they were drawn randomly from the population. The most likely pair of parents will be compared to the second most likely and the difference in LOD scores (Δ LOD) was calculated. The

Table 3 – Microsatellite loci to be used for parentage analysis. Alleles and H_o are the number alleles and the observed heterozygosity, respectively, typical of each locus for samples of steelhead from the Snake River Basin (Paul Moran, unpublished data).

Locus	Alleles	Ho	Reference
Ocl1	25	0.793	Condrey and Bentzen (1998)
Ogo4	16	0.818	Olsen et al. (1998)
Oke4	22	0.806	Buchholz et al. (2001)
Oki23MMBL	23	0.770	#AF272822
Omy71	21	0.815	K. Garbi, pers. comm.
Omy77	24	0.863	Morris et al. (1996)
Omy1001	32	0.877	P. Bentzen, pers. comm.
Omy1011	20	0.880	P. Bentzen, pers. comm.
Oneu14	12	0.583	Scribner et al. (1996)
Ots1	31	0.805	Banks et al. (1999)
Ots3	12	0.669	Banks et al. (1999)
Ots4	10	0.729	Banks et al. (1999)
Ots100	27	0.814	Nelson and Beacham (1999)
Ssa289	10	0.452	McConnell et al. (1995)
Ssa407	27	0.849	Cairney et al. (2000)
Ssa408	23	0.879	Cairney et al. (2000)

simulation function of the FAMOZ program will be used to generate expected distributions of Δ LOD scores for correct and incorrect assignments. The type of analysis will also be used to identify parents separately within each sex, in order to evaluate the contributions of (unsampled) resident males. As an alternative method of fitness estimation, we will also use the FAMOZ program output to fractionally assign progeny to the 20 most likely parent pairs in proportion to their likelihoods. In situations where multiple parent pairs are compatible with some offspring, fractional assignment methods also provide a statistically robust way to estimate selection gradients and relative reproductive success (e.g., Morgan and Conner 2001; Nielsen et al. 2001; Murdoch et al. 2007).

Selection and fitness analysis – Absolute fitness (progeny counts) within sexes will be converted to relative fitness by dividing by the mean fitness. The effects of age, origin, weight and run timing on fitness will be estimated using selection gradient analysis (Lande and Arnold 1983). All statistical analyses will be conducted using the general linear model (GLM) function in the R computer package. We will estimate the effects of the following traits (all measured at Tumwater Dam) on fitness: weight, run time, age, and origin (hatchery or wild). Weight will be cube root transformed prior to standardization, and run time will be converted to ordinal days. Traits will be standardized within each sex by subtracting the mean and dividing by the standard deviation.

Resident trout – Previous pedigree studies of steelhead have typically found that a proportion of progeny cannot be assigned to any anadromous parents, and that the non-assignment is biased such that the missing parent is more likely to be a male than a female (Seamons 2004; Seamons et al. 2004; Araki et al. 2007a; Araki et al. 2007b). These studies have concluded that resident trout males commonly mate with anadromous females, thus producing offspring whose male parents were not sampled during the spawning migration. We anticipate a similar issue in the Wenatchee, and plan to deal with it in two ways. First, we will estimate parentage separately for each sex to determine if progeny are more likely to have an identified female parent than male parent. Second, to the degree possible, we will attempt to sample potential resident spawners. These will include the juvenile parr samples we obtain in the normal course of the study (see above), as well as samples of released hatchery fish, which have the potential to residualize.

Task 4 – Conduct ecological studies to examine differences in life history traits of hatchery and naturally produced steelhead

Difference in reproductive may arise through genetic causes (domestication), environmental (hatchery practices), or their interaction. Determining the source of any observed difference is rarely reported. We propose to replicate the approach developed by Araki et al. (2007b) to determine if differences in reproductive success are largely genetic based. Tissue samples from two previous hatchery broodstock generations (i.e., parents and grand parents) of the 2008 brood (1st generation 2004 and 2005 broods; 2nd generation 2000 – 2002 broods) will be analyzed. If the relative difference in fitness among generations is similar to that observed between hatchery and naturally produced fish, we would conclude that genetic factors are largely responsible for the observed difference.

We also proposed conducting ecological studies (2010 and 2011) designed to determine to what extent hatchery practices (e.g., spawn timing) or hatchery operations (e.g., release location) have contributed to any observed difference in relative reproductive success. The implementation of such studies will be consistent with the methods already approved by the HCP HC. Comparisons of naturally produced and hatchery traits will be made based on data collected in the hatchery or natural environment. In addition to those traits examined using the selection gradient analysis, we will also collect data on traits (Table 4) that could influence reproductive success consistent with the methods outlined in Murdoch et al. (2006) where applicable.

Table 4. Life history traits that will be measured to determine their influence on the relative reproductive success of natural and hatchery produced steelhead in the upper Wenatchee Basin.

Trait	Metric	Location
Spawn timing	Day of the year	Natural
Spawn location	River kilometer	Natural
Redd microhabitat		
Redd shape	Length/width	Natural
Redd size	Square meters	Natural
Redd morphology	Various locations (cm)	Natural
Water velocity	Various locations (m/s)	Natural
Water depth	Various locations (cm)	Natural
Redd mesohabitat		
Habitat type	Categorical	Natural
Channel morphology	Categorical	Natural
Location within channel	Distance to bank/total width (m)	Natural
Distance to nearest redd	Meters	Natural
Emergence date	TU’s to estimated emergence	Natural
Fecundity	Number of eggs	Hatchery
Egg size	Weight (g)	Hatchery
Sex ratio	Males per female	Natural

Reporting Results and the Adaptive Management Process

The first comparison of relative reproductive success of hatchery and natural produced steelhead will be after samples from age-1 parr are analyzed in 2010 (2008 brood). We propose to host an annual one-day workshop to discuss results of the study. The objective of the workshop is to inform the HCP HC of recent findings and discuss possible outcomes and management scenarios. Because no ecological data was collected for the first two brood years, the first opportunity to discuss both genetic and ecological data will be after parr from the fourth brood year has been analyzed in 2013 (Figure 7). A second important time period would be after the smolt life history analysis is complete in 2015. Workshops held prior to these dates will provide an opportunity to discuss our results in context of other studies (i.e., Hood River and Kalama) and understand how the results relate to pending changes in the Wenatchee steelhead hatchery program. We consider this an important step in ensuring the results of the study are incorporated into the future program.

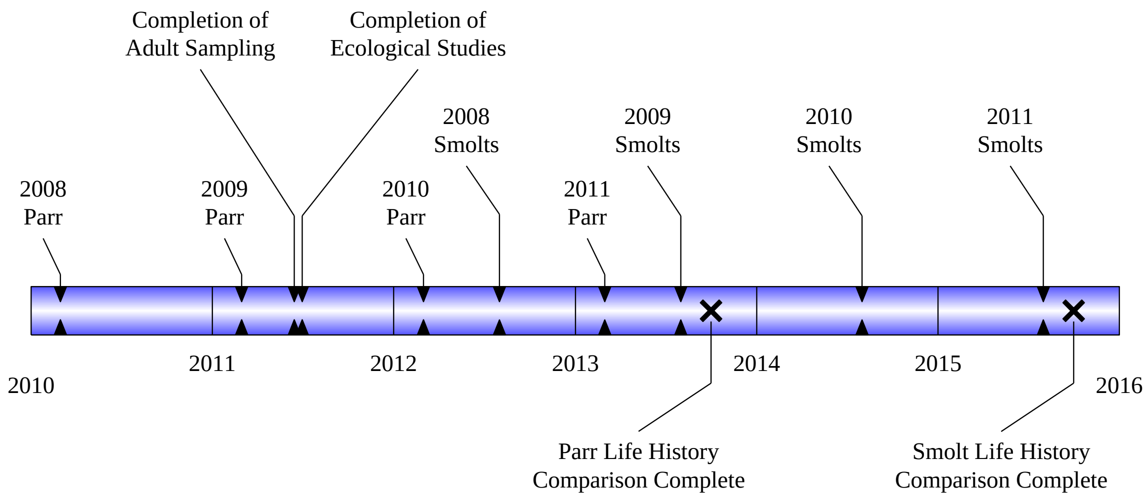

Figure 7. Proposed timeline for the completion of major milestones in the Wenatchee Steelhead Relative Reproductive Success Study.

Collaboration with Existing Activities

The proposed study design requires an increase in capture and handling of adult steelhead at Tumwater Dam, but no increase in "take" will be required for the collection of juvenile tissues samples due to the extensive monitoring ongoing in the Wenatchee Basin.

Spring Chinook Salmon Relative Reproductive Success Study

An important aspect of this proposal is that we propose to leverage a large amount of the data collection from activities/studies currently being implemented in the Wenatchee Basin. Operation of Tumwater Dam is already funded from May through July as part of similar reproductive success study on spring Chinook salmon. Steelhead captured during this time period will be sampled in a manner consistent with the study objectives.

Integrated Status and Effectiveness Monitoring Program (ISEMP)

The ISEMP currently funds, in part, activities (i.e., smolt traps and remote PIT tagging) that will provide most of the necessary juvenile steelhead samples for the study. Both Wenatchee River smolt traps and the Nason Creek smolt trap are partially funded through the ISEMP. The ISEMP also funds the remote PIT tagging in the Wenatchee Basin, which will be how tissue samples from age-1 parr are collected for the study and provide PIT tags for the all the smolt traps.

Wenatchee Coho Reintroduction Program

The coho reintroduction program in Nason Creek partially funds the operation of the smolt trap. Tissue samples from steelhead smolts capture at the trap will be included in the genetic analysis.

Grant County PUD

The smolt trap in Nason Creek is also partially funded by Grant County PUD. Tissue samples from steelhead smolts capture at the trap will be included in the genetic analysis. Steelhead parr captured at the White River smolt trap, funded by Grant PUD, may also be included in the genetic analysis.

Chelan County PUD

Chelan County PUD currently funds the operation of the smolt trap in the Chiwawa River and partially funds both traps in the Wenatchee River. Tissue samples from steelhead smolts capture at these traps will be included in the genetic analysis. Chelan County PUD also provides personnel to PIT tag juveniles at the trap sites and during remote tagging activities. PIT tagging of juvenile steelhead is critical in preventing the double sampling of individuals.

Shared Objectives Consistent with Chelan PUD M & E Program

In 2005, the Rock Island Hatchery Committee adopted and approved a formal monitoring and evaluation plan (Murdoch and Peven 2005). Within the Plan, the relative reproductive success of steelhead was specifically addressed as a separate study that will be included in the Plan. While the proposed study plan is consistent with the M & E Plan, data generated and analyzed under the proposal could also be directly incorporated into the M & E Plan. Below, we describe how data collected under this proposal specifically relates to the M & E Plan and fills critical data gaps in existing M & E activities.

Objective 2: Determine if the run timing, spawn timing, and spawning distribution of both the natural and hatchery components of the target population are similar.

All of the metrics required for this objective will be collected during the ecological studies, if implemented in 2010 (Table 6). Survey frequency of high density spawning areas should be increased (i.e., twice a week) to ensure sample sizes are adequate and will required additional funding. Under the current proposal, data will only be collected for two years. However, we hope to develop methods for longer term monitoring for steelhead spawn timing and location required in the M & E Plan.

Objective 3: Determine if genetic diversity, population structure, and effective population size have changed in natural spawning populations as a result of the hatchery program. Additionally, determine if hatchery programs have caused changes in phenotypic characteristics of natural populations.

Data for life history traits identified in this objective will also be collected during the ecological studies starting 2010 (Table 6). Furthermore, 50% of the genetic samples required for the genetic analysis in the M & E Plan will be analyzed as part of the reproductive success study (Appendix A). Additional cost savings may be realized in the future when the genetic objectives in the M & E Plan are reanalyzed (i.e., every 10 years), some tissues samples will have already been analyzed as part of the reproductive success study.

Significant overlap does exist among the proposed reproductive success study and the M & E Plan. During the course of the study, much of the data required for objective 2 and 3 in the M & E Plan will be collected. Knowledge and field methods developed during the study may also guide less intensive monitoring required for long term monitoring of this population.

Table 6. Comparison of data collected or analysis under the proposed steelhead reproductive success study and data collected or analysis required under the Chelan PUD M & E Plan.

Activity	**M & E Plan**		**RSS**	**Comments**
	Current	**Required**		
Ecological studies				
Run timing	X		X	Primary metric in Obj. 2
Spawn timing		X	X	Primary metric in Obj. 2
Spawn location		X	X	Primary metric in Obj. 2
Redd microhabitat			X	RSS only
Redd mesohabitat			X	RSS only
Age composition	X		X	Primary metric in Obj. 3
Size at age	X		X	Primary metric in Obj. 3
Fecundity	X		X	Secondary metric
Sex ratio	X		X	Secondary metric
Genetic analysis				
Parental assignments			X	RSS only
Allele frequency		X		Primary metric in Obj. 3
Genetic distance within population		X		Primary metric in Obj. 3
Genetic distance b/w populations		X		Primary metric in Obj. 3
Effective population size		X		Primary metric in Obj. 3

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Appendix A. Estimate number of tissue samples required for conducting the relative reproductive success study and M & E objectives. Bold italics numbers are those samples that will be analyzed under the reproductive success study, but will also be used in analysis for the M & E Plan.

Adult steelhead samples collected				**Samples analyzed**		**Year of analysis**
Brood	**Source**	**Wild**	**Hatchery**	**M & E Plan**	**RSS**	
1998	Broodstock	35	36	71		2009
1999	Broodstock	36	65	101		2009
2000	Broodstock	36	65	***101***	101	2009
2001	Broodstock	50	101	***151***	151	2009
2002	Broodstock	95	64	***159***	159	2009
2003	Broodstock	49	90	139		2009
2004	Broodstock	75	61	***136***	136	2009
2005	Broodstock	87	100	**187**	187	2009
2006	Broodstock	93	69	162		2009
2007	Broodstock	77	65	142		2009
2008	Broodstock	75	56	***131***	131	2009
2008	Tumwater	513	888	***144***	1,401	2009
2009	Broodstock	100	100	192		2009
2009	Tumwater	400	800	***144***	1,200	2009
2010	Broodstock	100	100	192		2010
2010	Tumwater	400	800	***144***	1,200	2010
2011	Broodstock	100	100	192		2011
2011	Tumwater	400	800	***144***	1,200	2011
2012	Tumwater	400		***144***	400	2012
2013	Tumwater	400		***144***	400	2013
2014	Tumwater	400		***144***	400	2014
2015	Tumwater	400		***144***	400	2015
2016	Tumwater	400		***144***	400	2016
2017	Tumwater	400		***144***	400	2017
	Total			3,496	8,266	

Juvenile steelhead samples collected				**Samples analyzed**		**Year of analysis**
Year	**Source**	**Parr**	**Smolts**	**M & E Plan**	**RSS**	
2007	Chiwawa		132	96		2009
	Nason		85	96		2009
	Wenatchee					
	Lower Wen.		144	96		2009
	Entiat		144	96		2009

	Peshastin					
2008	Chiwawa		144	96		2009
	Nason		139	96		2009
	Wenatchee					
	Lower Wen.		144	96		2009
	Entiat		86	96		2009
	Peshastin	144		96		2009
2009	Chiwawa	500	144	*96*	250	2009
	Nason	500	144	*96*	250	2009
	Wenatchee	500			250	2009
	Lower Wen.		144	*96*		2009
	Entiat		144	96		2009
	Peshastin	144		96		2009
2010	Chiwawa	500	500	***96***	500	2010
	Nason	500	500	***96***	500	2010
	Wenatchee	500			250	2010
	Lower Wen.		500	***96***	250	2010
	Entiat		144	96		2010
	Peshastin	144		96		2010
2011	Chiwawa	500	500	***96***	500	2011
	Nason	500	500	***96***	500	2011
	Wenatchee	500			250	2011
	Lower Wen.		500	***96***	250	2011
	Entiat		144	96		2011
	Peshastin	144		96		2011
2012	Chiwawa	500	500		500	2012
	Nason	500	500		500	2012
	Wenatchee	500			250	2012
	Lower Wen.		500		250	2012
2013	Chiwawa		500		250	2013
	Nason		500		250	2013
	Wenatchee					
	Lower Wen.		500		250	2013
2014	Chiwawa		500		250	2014
	Nason		500		250	2014
	Wenatchee					
	Lower Wen.		500		250	2014
	Total			2,304	6,750	
	Grand total			5,800	15,016	
	M & E only			2,919	50%	